How to formulate membrane potential in a spatially homogeneous myocyte model?

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Abstract

Membrane potential in a mathematical model of a cardiac myocyte can be formulated in different ways. Assuming a spatially homogeneous myocyte that is strictly charge-conservative and electroneutral as a whole, two methods will be compared: (1) the differential formulation $dV/dt = -I/C_m$ of membrane potential used traditionally; and (2) the capacitor formulation, where membrane potential is defined algebraically by the capacitor equation $V = Q/C_m$. We examine the relationship between the formulations, assumptions under which each formulation is consistent, and show that the capacitor formulation provides a transparent, physically realistic formulation of membrane potential, whereas use of the differential formulation may introduce unintended and undesirable behavior, such as monotonic drift of concentrations. We prove that the drift of concentrations in the differential formulation arises as a compensation for failure to assign all currents in concentrations. As an example of these considerations, we present an electroneutral, explicitly charge-conservative formulation of Winslow et al. model (1999), and extend it to describe membrane potentials between intracellular compartments.

1   Introduction

The single most important variable in an electrophysiological whole-cell model is membrane potential, defined as the potential difference across the cell membrane. It drives both gating of ion channels and fluxes of ions through the membrane. The basis for electrophysiological single-cell modeling was first provided by the Hodgkin-Huxley model of the neuronal action potential (1952). In their model, membrane potential was defined by an ordinary differential equation in which the time-derivative of membrane potential equals the sum of all ion currents through the membrane divided by membrane capacitance. Their model

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assumed constant ionic concentrations and dynamic concentrations were added in later models (DiFrancesco and Noble, 1985). While tracking of concentrations made the models more realistic, doing so introduced new problems, including drift of concentrations under repeated stimuli (see Fig. 1), over-determined initial conditions and an infinite number of steady states (Guan et al., 1997; Hund et al., 2001; Kneller et al., 2002).

Long-term drift of concentrations is present in the DiFrancesco-Noble (Guan et al., 1997) and Luo-Rudy models (Luo and Rudy, 1994). Using numerical simulations, Hund et al. (2001) showed that the Luo-Rudy model has a steady state and no concentration drift, when it is assumed that the externally applied stimulus current is carried by potassium (K^+) ions and that this ion flux contributes to the rate of change in intracellular K^+ concentration. Similarly, Kneller et al. (2002) demonstrated that this finding also holds true in their model. However, neither the reason for nor the origin of ion concentration drift has been addressed quantitatively. In this study, we explain the mechanism of concentration drift (Sec. 3.1) and examine the number of steady states present in the above models (Sec. 3.2).

We also investigate alternative formulations of membrane potential which preserve charge-conservation and electroneutrality. In particular, we compare what we refer to as the differential and capacitor formulations of membrane potential (Sec. 2), and consider issues affected by the formulation of membrane potential (Sec. 3). Two examples (Sections 4 and 5) show how membrane potential may be formulated rigorously in a computational model of the cardiac ventricular myocyte developed by Winslow et al. (1999; abbreviated WRJ). Our results show that the capacitor formulation provides a transparent, well-defined formulation of membrane potential in a spatially homogeneous myocyte model.

2 The myocyte as a capacitor

2.1 Formulation of membrane potential

Following Hodgkin and Huxley (1952), a spatially homogeneous single-cell model is described as a parallel RC-circuit model. Given an initial value, membrane potential V is then defined by the differential equation

$$\frac{dV}{dt} = -\frac{1}{C_m}I, \tag{1}$$

where I is the sum of all outwardly directed membrane currents and C_m is total membrane capacitance. This formulation of membrane potential is referred to as the *differential formulation*.

Membrane potential can be defined by assuming that a cell is a capacitor, consisting of an ideal conductor representing the interior of the cell, a dielectric representing the ability of the cell membrane to separate charge and a second ideal conductor representing the extracellular space surrounding the cell. In this *capacitor formulation*, membrane potential is defined as the ratio of charge Q

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to total membrane capacitance C_m:

$$V = Q/C_m. \tag{2}$$

Charge Q includes contribution from all charged particles in a cell. More specifically, membrane potential of a model containing a single intracellular compartment with N ion species $\{S_k\}_{k=1}^N$ is given by

$$V = \frac{F v_{\mathrm{myo}}}{C} \sum_{k=1}^{N} z_k [S_k]_i, \tag{3}$$

where z_k is the valence of species S_k, v_{myo} is volume of myoplasm and F is Faraday's constant.

A biophysically detailed model often describes a myocyte using more than two compartments. The capacitor formulation (2) is extended to multiple compartments by describing the cell as a network of capacitors (Fig. 2A), where each dielectric corresponds to an interface between two compartments. The interior of a compartment is considered to be an ideal conductor insulated from other compartments by a membrane. The membrane potentials can be expressed as functions of charges of compartments and of capacitances of interfaces. In particular, a compartment that is completely enclosed within a larger compartment influences the membrane potential of the surrounding compartment only via charge, geometry is irrelevant (Griffiths, 1989). For example, Figures 2A and 2B show graphical and capacitor representations of a three-compartment cell model. Membrane potentials are given by $V_1 = (Q_{\mathrm{myo}} + Q_{\mathrm{mito}})/C_1$, and $V_2 = Q_{\mathrm{mito}}/C_2$, each depending only on net charge of the enclosed volume.

2.2 Electroneutrality and homogeneous concentrations

Membrane potential is generated by a small number of ions, the bulk ionic concentration is electroneutral (Hille, 2001). We require that (1) a model is electroneutral as a whole, that is, the net charge is zero; and that (2) the concentrations are spatially homogeneous (i.e. well-mixed) in each compartment. Since such requirements are not necessarily satisfied in the presence of charged particles, we will show this to be the case for the model presented here.

In an ideal conductor, induced charges balance electric field in such a way that potential is constant inside the conductor. Hence the interior of each compartment is exactly electroneutral in the capacitor approximation and all net charge is located on the compartment boundary. This is consistent with narrow physiological range of membrane potentials, which also requires that sum $\sum z_k[S_k]_i$ must be nearly zero.

Both anions and cations must be present in each compartment, since the absence of anions (cations) would imply that all cations (anions) are located on the membrane. The concentration of ions that comprise the induced charge is extremely low compared to bulk concentrations and can typically be neglected. For example, in the WRJ model, range $[-100, 50]$ mV limits induced charge

to correspond to a monovalent ion concentration in range $[-0.5, 0.25]$ μmol/L, which is negligible compared to most intracellular ion concentrations. Electroneutrality of the interior allows the assumption that ion concentrations are homogeneous in each compartment.

In a typical experimental setup, a myocyte is embedded in an electroneutral solution of essentially infinite volume relative to the cell volume. While this extracellular space (ES) is treated like any other compartment in a cell model, the assumption of infinite volume (implied by constant extracellular concentrations) brings certain extra complications that will be addressed in the following. If the myoplasm has charge Q, charge of the ES is $-Q$. However, if the charge density of ES, $F \sum_{k=1}^{N} z_k [S_k]_e$ (notation as in (3)), is non-zero, then the ES has infinite charge which implies infinite membrane potential. Hence, the ES must have *zero* charge density, but typically *non-zero* charge. The paradox arises from infinite volume: if the charge of the ES is finite, the average charge density is zero because no finite flow of ions can change the concentrations within an infinite volume eventhough charge is altered. As with all other compartments, all net charge is located on the boundary of the ES, and the remainder of the compartment is exactly electroneutral. In conclusion, the capacitor approximation of the cell is consistent with the requirement of electroneutrality and the assumption that ions are homogeneously distributed in a compartment.

2.3   Connection between the formulations

Next, we will study the exact connection between the two formulations of membrane potential. In the differential formulation, membrane potential is defined as an independent variable and does not depend on ion concentrations, whereas in the capacitor formulation, membrane potential is a function of concentrations. Hence, in the differential formulation initial conditions are over-determined, since initial conditions are assigned independently for interdependent variables. This issue can be resolved by introducing implicitly-defined ion concentrations in the differential formulation, as will be shown in the following.

For example, assume a one intracellular compartment model with M concentrations $\{[S]_m\}_{m=1}^{M}$ and N currents $\{I_k\}_{k=1}^{N}$, is defined in the differential formulation by

$$
\frac{dV}{dt} = -\sum_{k=1}^{N} I_k / C_m,
$$

$$
\frac{d}{dt}[S]_m = -\sum_{l=1}^{N_m} I_{a_{m,l}} / (z_m F v),
$$

(4)

where v is the volume of the compartment, $a_{m,l}, l = 1, ..., N_m$, indexes the N_m currents assigned to ion species S_m of valence z_m.

Assume that not all currents carry ion species modeled by any of the intracellular concentrations $[S]_m$. Such current are assigned to an implicitly-defined

monovalent concentration $[S]_I$,

$$\frac{d}{dt}[S]_I = -\sum_{l=1}^{N_I} I_{a_{I,l}}/Fv, \qquad (5)$$

where $N_I = N - \sum_m N_m$. Combining equations (4) and (5) yields

$$\frac{d}{dt}V = Fv\Big(\sum_m \frac{d}{dt}\big(z_m[S]_m\big) + \frac{d}{dt}[S]_I\Big)/C_m. \qquad (6)$$

Integrating equation (6) gives membrane potential

$$V = Fv\Big(\sum_m z_m[S]_m + [S]_I\Big)/C_m. \qquad (7)$$

The initial value of $[S]_I$ is determined by the assumption regarding initial charge density. Equation (7) shows the exact connection of the differential formulation (4) to the capacitor formulation (2) (in the two-compartment case, but the derivation can be generalized to any number of compartments). The major difference between the formulations is $[S]_I$, which accounts for both the currents missing from equation (4) and the charge missing from initial conditions in the differential formulation. The formulations are equivalent if $[S]_I$ is constant and all movement of charge is captured by the currents. Concentration $[S]_I$ and its time evolution are the source of a variety of problems, as will be shown in the following.

3 Consequences of membrane potential formulation

In this section, we will show how the physical constraints presented above influence the formulation of a cell model.

3.1 Concentrations and drift

Here we examine how and under what conditions spurious concentration drift arises in the differential formulation. Assume a model with K^+ concentration $[K]_i$ and a constant anion concentration $[B]_i$, in which membrane potential is given by

$$Fv_{\mathrm{myo}}([K]_i - [B]_i)/C_m. \qquad (8)$$

It is clear that stimulus current I_{stim} must influence $[K]_i$ if it is to modify membrane potential. Charge-conservation and electroneutrality require that the stimulus current originates from the ES.

In the differential formulation, stimulus current has not historically been assigned to a particular ion concetration. Formulating the above model in this

manner yields a model comparable to the one defined above defined by

$$\frac{dV}{dt} = -\frac{1}{C_m}(I_K + I_{\text{stim}}),$$
$$\frac{d[\text{K}]_i}{dt} = -\frac{1}{Fv_{myo}}I_K,$$
(9)

where I_K is K^+ current through the cell membrane. Note that concentration $[\text{B}]_i$ does not appear in (9). If we assign the stimulus current to implicitly-defined anion concentration $[\text{E}]_i$ (no information on valence of species E is contained in equation (9)),

$$\frac{d[\text{E}]_i}{dt} = \frac{1}{Fv_{myo}}I_{\text{stim}},$$
(10)

and equation (7) implies that

$$V = Fv_{\text{myo}}([\text{K}]_i - [\text{E}]_i)/C_m.$$
(11)

Since all currents are explicitly accounted for in the concentrations, equation (11) shows that the model defined by equation (9) is charge-conservative, when concentration $[\text{E}]_i$ is included in the formulation. A positive stimulus current decreases $[\text{E}]_i$ monotonically and eventually makes it negative, that is, $[\text{E}]_i$ has spurious drift. To keep membrane potential in the physiological range, the decrease of $[\text{E}]_i$ must be compensated for by a decrease of $[\text{K}]_i$. Drift occurs in both the capacitor (equation 11) and differential formulations (equation 9) of the model, however, the cause of drift is transparent only in the capacitor formulation.

Explanation of the drift presented above may be generalized to any number of concentrations. In a model with unassigned current I_c, membrane potential is given by equation (7). The current I_c either increases or decreases $[\text{S}]_I$ monotonically. To keep membrane potential in the physiological range, charge density $\sum_{m \neq I} z_m[\text{S}]_m$, must compensate for the change in $[\text{S}]_I$, which is manifested as a monotonic drift of at least one of the concentrations $[\text{S}]_m$. Even a minor drift in a single ion concentration will influence membrane potential if it goes uncompensated. Hence drift is inevitable if there exists a current that transports charge and hence affects membrane potential, but does not contribute to any ion concentration. This is consistent with previous numerical studies (Hund et al., 2001; Kneller et al., 2002). Previously presented descriptions of the drift (e.g., Hund et al. (2001)) have stated that lack of charge conservation is at the root of the problem but did not demonstrate how drift arises. As shown above, concentration drift is a compensation for the change in the implicitly-defined concentration $[\text{S}]_I$.

How does the drift depend on pacing rate? Since drift is relative to the charge transported by the stimulus current, higher pacing rate leads to more rapid decrease of $[\text{E}]_i$, and consequently to faster drift in $[\text{K}]_i$, consistent with numerical simulations of Hund et al. (2001).

3.2 Steady state and time scales

An infinite number of steady states were observed in simulations of Guan et al. (1997), when initial concentrations were varied. If voltage is kept constant but concentrations are changed in the differential formulation, concentration $[S]_I$ (equation (7)) is changed (Sec. 3.1). If $[S]_I$ remains constant during a simulation, different values of $[S]_I$ result in different steady states. Hence, an infinite possible number of initial values of $[S]_I$ yield an infinite number of steady states. On the other hand, $[S]_I$ is not constant if any of the currents implicitly assigned to $[S]_I$ is non-zero, in which case the model may not have a steady state. In the capacitor formulation, $[S]_I$ is explicitly included in the initial conditions, which resolves this issue.

The time scale of exponential relaxation to steady state can be described by time constant τ, measured by fitting $A + Be^{-t/\tau}$ to either the diastolic voltage, Na^+ or K^+ concentrations (Fig. 3). The time constant is mainly determined by the balance of Na^+ and K^+ concentrations. This time constant divides the model time scales into two regimes: slow ($t > \tau$) and fast ($t < \tau$). The fast times scale is on the order of a few seconds or less, whereas the slow time scale is on the order of tens or hundreds of seconds. In a study of fast time scale processes, Na^+ and K^+ concentrations can be clamped. However, if concentrations are clamped for a time period comparable to τ, incorrect behavior will occur since implicit concentrations will change significantly during the simulation. Analogously, in voltage clamp a control current holds the membrane potential at the desired level. If this current is not assigned to any concentration, it is accounted for in implicit concentration and the behavior of the system will be incorrect in protocols with duration comparable to the time constant.

3.3 Rapid equilibrium approximation

The rapid equilibrium approximation (REA) often provides a powerful method to simplify a cell model (see, e.g., Hinch et al. (2004)). However, the REA requires instantaneous movement of charge in the model. Due to currents associated with instantaneous movement of charge, the differential and capacitor formulations may yield different results, as will be shown in the following.

Assume a model consists of a small-volume intracellular compartment and an infinite-volume extracellular compartment which are connected by a single ion channel that is described as a two-state Markov model. The intracellular compartment has anion concentration $[A]_i$ equal to extracellular K^+ concentration $[K]_o$. Also assume that the intracellular K^+ concentration $[K]_i$ is an instant function of the state of the channel (essentially a REA): if the channel is closed $[K]_i = 0$, otherwise $[K]_i = [K]_o$. Membrane potential $V = F([K]_i - [K]_o)/C_m$ depends on state of the ion channel. Assuming that α and β are opening and closing rates, respectively, of the ion channel, the probability P_C that the ion channel is closed evolves according to equation $dP_C/dt = \beta - (\alpha + \beta)P_C$.

Assume a large ensemble of N intracellular compartments described above to be located within a single cell. According to capacitor formulation, membrane

potential of the cell is then $V(t) = -NFP_C(t)[\text{K}]_\text{o}/C_m$. Since each individual channel allows non-zero flux only at the time of channel closing and opening, the total current seems to mostly be zero. However, the ensemble current through the cell membrane due to REA is $I = F(\beta - (\alpha + \beta)P_C)[\text{K}]_\text{o}N$. This current is missed in the naive application of the differential formulation, which thus yield incorrect membrane potential.

In a recent reformulation of the WRJ model by Greenstein et al. (2005, submitted to Biophys. J.), the REA was employed. In this case, current due to the REA is not easily solved from the equations, and use of the differential formulation introduces systematic, albeit small, error in calculation of the membrane potential (Fig. 1D-E). The small magnitude of this error is due to the small ratio of the diadic volume to the total cell volume. When a similar approximation is applied to a larger compartment, the difference can be significant.

4   Case study 1: the WRJ model

The WRJ model describes intracellular Ca^{2+} dynamics of the cardiac ventricular myocyte. The model consists of four intracellular compartments: myoplasm, network sarcoplasmic reticulum (NSR), junctional sarcoplasmic reticulum (JSR) and the diadic space (SS). The myocyte is embedded in the extracellular space containing constant ion concentrations. The WRJ model has concentration drift and consequently does not have a steady state; it does not exhibit physiologically realistic dependence of APD on pacing rate, nor is it electroneutral. As an application of the considerations in the previous two sections, we reformulate the WRJ model and address these issues in the following.

To address the above mentioned issues, we modified the model of (Mazhari et al., 2002) which was based on the WRJ model in the following manner: stimulus current I_stim is assigned to intracellular K^+ concentration $[\text{K}]_\text{i}$; conductances of background calcium current $I_\text{Ca,b}$, background Na^+ current $I_\text{Na,b}$ and Na^+-K^+ pump I_NaK were adjusted to balance concentrations in the long term; and K^+, Na^+ and stationary anion concentrations were added to compartments if not already present. We reformulated membrane potential using the capacitor formulation, in which membrane potential is given by

$$V = [Q_i + Q_\text{NSR} + Q_\text{JSR} + Q_\text{SS}]/C_m, \tag{12}$$

where charges Q_k are defined through

$$\begin{aligned}
Q_i &= Fv_\text{myo}([\text{K}]_\text{i} + [\text{Na}]_\text{i} + 2[\text{Ca}]_\text{i}^\text{tot} - [S]_i), \\
Q_\text{NSR} &= Fv_\text{NSR}([\text{K}]_\text{NSR} + [\text{Na}]_\text{NSR} + 2[\text{Ca}]_\text{NSR} - [S]_\text{NSR}), \\
Q_\text{JSR} &= Fv_\text{JSR}([\text{K}]_\text{JSR} + [\text{Na}]_\text{JSR} + 2[\text{Ca}]_\text{JSR}^\text{tot} - [S]_\text{JSR}), \\
Q_\text{SS} &= Fv_\text{SS}([\text{K}]_\text{SS} + [\text{Na}]_\text{SS} + 2[\text{Ca}]_\text{SS}^\text{tot} - [S]_\text{SS}),
\end{aligned} \tag{13}$$

where v_k is volume of compartment k, concentration $[S]_k$ is static anion concentration in compartment k. The index tot refers to the sum of buffered and free Ca^{2+}.

In a ventricular myocyte, action potential duration (APD) depends on pacing rate. Figure 1A shows steady state action potentials at pacing rates 0.25-2 Hz, with higher pacing rates producing shorter action potentials. The pacing rate dependence of APD arises mainly as a result of long-term changes in $[Na]_i$ and $[K]_i$. Figure 1B shows simulations started (1) with intracellular concentrations set to extracellular concentrations; (2) from a minimally perturbed steady state (static charges unchanged); and (3) with steady state $[Na]_i$ and $[K]_i$ replaced by $[Na]_i + 1$ and $[K]_i - 1$. In each case, limit cycles in $(V, [Na]_i)$ phase space are identical within numerical accuracy, showing that the steady state is unique, given parameters including pacing rate.

Figure 3 illustrates long-term changes in the model, sampled at 1 Hz. The simulation is started from a state with no concentration gradients, Figure 3A shows diastolic membrane potential. Figure 3B exhibits the increase of $[Na]_i$ (initially set to $[Na]_o$) and the decrease of $[K]_i$ (initially set to $[K]_o$) to their steady state values. Homeostasis is approached approximately exponentially with a time constant of 90 seconds. Figure 3C shows the decrease of diastolic $[Ca]_i$ towards its steady state. Two oscillatory regimes of $[Ca]_i$ emerge: first at roughly 800 seconds with small, subthreshold oscillations; second with large amplitude oscillations at roughly 1,400 seconds. Figure 3D shows the non-trivial time evolution of APD during the simulation.

5 Case study 2: Compartmental membrane potentials

Intracellular compartments are important for proper myocyte function. In particular, the sarcoplasmic reticulum (SR) stores Ca^{2+} ions. While the process of uploading of Ca^{2+} into the SR is electrogenic, it does not seem to influence SR membrane potential that is observed to be small in amplitude (v_{SR}; Bers, 2001). Pure diffusion of ions through SR membrane cannot explain v_{SR}, since it would balance ion species separately without consideration to v_{SR}. A small v_{SR} requires that movement of counter-ions, likely K^+ and Cl^-, balances the potential difference generated by Ca^{2+} movement (Pollock et al., 1998; Kargacin et al., 2001). Indeed, SR membrane is known to have Cl^- channels gating according to v_{SR} (Townsend and Rosenberg, 1995), suggesting that v_{SR} does affect Ca^{2+} handling unless kept at almost zero voltage. Somewhat counter-intuitively, zero v_{SR} requires currents driven by v_{SR}.

5.1 Formulation

To better understand the basis for v_{SR} and the concentrations of ions in SR, we extend the model of Case study 1 to describe intracellular membrane potentials. In this case, the cell is described as a network of capacitors shown in Fig. 2B. Membrane potential V_k of interface k (Fig. 2B) can be expressed as a function

of capacitance C_k of and charge q_k bound to interface k by

$$V_k = q_k/C_k. \tag{14}$$

Given net charges $Q_m = Fv_m \sum_l z_l[S_l]_m$, the charges q_k are

$$
\begin{aligned}
q_1 &= \frac{-\beta(b + C_5 s + Q_{\text{JSR}}) - Q_{\text{NSR}}/C_7 - s}{r + \beta(1 + C_3\gamma + C_5 r)}, \\
q_2 &= -q_1 - Q_e, \\
q_3 &= q_1 C_3/C_1 - q_2 C_3/C_2, \\
q_4 &= q_2 - q_3 - Q_{\text{SS}}, \\
q_5 &= q_3 C_5/C_3 - q_4 C_5/C_4, \\
q_6 &= Q_{\text{JSR}} - q_4 + q_5, \\
q_7 &= -q_6 - Q_{\text{NSR}},
\end{aligned}
\tag{15}
$$

where $\beta = \frac{1}{C_6} + \frac{1}{C_7}, \gamma = \frac{1}{C_1} + \frac{1}{C_2}, b = Q_{\text{SS}} + Q_e(1 + \frac{C_3}{C_2}), r = [1 + \gamma(C_3 + C_4)]/C_4, s = (Q_e(C_4 C_2 + C_2 C_4 + C_3) + C_2 Q_{\text{SS}})/(C_4 C_2)$. Charge Q_e is given by electroneutrality, $Q_e = -(Q_m + Q_{\text{JSR}} + Q_{\text{NSR}} + Q_{\text{SS}})$.

An ion channel senses voltage V across the membrane in which the channel is located. Then the flux J_G of ions of species S between compartments a and b is given by the Goldman equation (Hille, 2001)

$$J_G = D\frac{zVF}{RT}\frac{[S]_a e^{zVF/RT} - [S]_b}{e^{zVF/RT} - 1}. \tag{16}$$

where D is the diffusion coefficient, z valence, T temperature and R universal gas constant. Ion channels (other than Ca^{2+} channels) between intracellular compartments are assumed to always be open and to be selective to a single ion species. In particular, myoplasm-SS and JSR-NSR interfaces have large-conductance, permanently-open pores. Each compartment contains Cl^- and stationary anion concentrations.

To include the effect of voltage V_7 modulating Ca^{2+} transport to NSR, we derived a model for the SERCA2a pump assuming Michaelis-Menten kinetics, that SERCA2a achieves equilibrium with Ca^{2+} instantaneously, and that rates α and β out of the intermediate Michaelis-Menten state are related by $e^{-\eta\Delta G/kT} = \alpha/\beta$, where $\eta\Delta G$ is free energy of ATP breakdown. The flux through SERCA2a is

$$J_{\text{SERCA}} = V_{max}\frac{[\text{Ca}]_i^2/K_i^2 - [\text{Ca}]_{\text{NSR}}^2/K_{NSR}^2}{1 + [\text{Ca}]_i^2/K_i^2 + [\text{Ca}]_{\text{NSR}}^2/K_{NSR}^2}, \tag{17}$$

where $K_{NSR} = K_i e^{-(\eta\Delta G_{ATP} - 4e_0 V_7)/kT}$, in which $\eta = 0.74$, e_0 is elementary charge, $K_i = 300$ nmol/L.

5.2   Results

The model has a realistic dependence of APD on pacing rate, as evidenced by Figure 4A. Intracellular membrane potentials are consistently small but non-zero (Fig. 4B). Membrane potentials V_3 and V_7 between myoplasm and SS and

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NSR are essentially zero. Membrane potentials V_4, V_5 and V_6 between JSR and other compartments are non-zero, roughly 1 mV, as a result of Ca^{2+} buffering by Calsequestrin in JSR, the absence of which would reduce these membrane potentials to essentially zero. External membrane potential of SS (V_2) tracks closely external membrane potential of myoplasm, V_1.

When diffusion rates between myoplasm and SR are reduced, intracellular membrane potentials are increased (Fig. 4C). The maximal NSR membrane potential that SERCA can overcome by employing energy of ATP hydrolysis (56 kJ/mol; Bers, 2001) is 237 mV. Without the movement of counter-ions, 7.5 μmol/L would be the maximal increase of $[Ca]_{NSR}$ over $[Ca]_i$, while the measured concentration difference is roughly 0.7 mmol/L (Bers, 2001). Since cells are sensitive to any disruption in Ca^{2+} handling, even a minor SR membrane potential has functional consequences.

Case study 2 demonstrates that (1) Intracellular membrane potentials can be incorporated in a cell model, and that (2) they give physical constraints on intracellular concentrations and require delicate balance of charges; (3) the magnitude of SR membrane potential v_{SR} can be explained by movement of counter-ions; and (4) buffering of Ca^{2+} affects JSR membrane potential.

6 Discussion

6.1 Which formulation is appropriate?

The capacitor formulation expresses membrane potential as a function of charge and capacitance, whereas the differential formulation uncouples membrane potential from concentrations. The main differences between the two formulations are an integration constant (the differential formulation is time derivative of the capacitor equation), and "independence" of membrane potential from concentrations in the differential formulation. These two issues imply the presence of a dynamic, implicitly-defined ion concentration(s) in the differential formulation (Sec. 3.1), which makes interpreting simulation results difficult and prone to errors, in addition to the presence of spurious drift of concentrations and issues with steady state. The differential formulation is equivalent to a particular capacitor formulation, which, however, may not be the one intended due to the presence of implicit concentrations.

The capacitor formulation requires "almost" electroneutrality and carefully chosen initial conditions due to sensitivity of membrane potential to net charge. However, these are physical constraints, since even a small additional charge can have a drastic effect on membrane potential. The capacitor formulation provides a transparent formulation of membrane potential, and requires no implicitly-defined concentrations. The differential formulation is best viewed as a shorthand notation for the more complete and better-defined capacitor formulation.

6.2 Comparison with previous studies

Guan et al. (1997) show that the DiFrancesco-Nobel model has infinite number of steady states, when initial concentrations are varied. To address this issue, they suggest that concentrations should be treated as parameters. However, they neglected the implied concentration in their study, inclusion of which resolves the issue. Consistent with our explanation (Sec. 3.1), Kneller et al. (2002) observed that if the sum of concentration changes in initial conditions is zero, the steady state stays the same.

The sinoatrial node model by Endresen et al. (2000) uses the capacitor equation for membrane potential. However, the reasoning behind their definition is different from ours. The membrane potential of a simplified model described in Sec. 3.1 is given by equation $Fv_{\mathrm{myo}}([\mathrm{K}]_{\mathrm{i}} - [\mathrm{B}]_{\mathrm{i}})/C_m$. Our interpretation of this equation is that concentration $[\mathrm{B}]_{\mathrm{i}}$ represents intracellular concentration of an anion concentration required for electroneutrality. In Endresen et al. (2000), $Fv_{\mathrm{myo}}[\mathrm{B}]_{\mathrm{i}}/C_m$ represents charge of the extracellular space, which however cannot be computed directly from the concentrations in the extracellular space due to its infinite volume.

Varghese and Sell (1997) derived a "latent conservation law" showing that membrane potential can be expressed as a function of concentrations. They showed that when each current is assigned to contribute to some concentration, the implicit concentration is constant. As they point out, the reason for emergence of the conservation law is best understood from the capacitor equation (2). However, they did not consider time-dependent changes in the implicit concentration, study of which is required to understand model phenomena such as concentration drift.

Using numerical simulations Hund et al. (2001) showed that assignment of stimulus current to $[\mathrm{K}]_{\mathrm{i}}$ is sufficient to remove concentration drift in the Luo-Rudy model (Luo and Rudy, 1994). They interpret the drift as a consequence of "non-charge-conservative" formulation of the model. However, we proved that a "non-charge-conservative" formulation is actually charge-conservative (Sec. 2.3), when the implicit concentrations is taken into account, even in the presence of spurious concentration drift (Sec. 3.1). In particular, this means that the equations defining the differential formulation require that the system is always charge-conservative. On the other hand, a model can be "non-charge-conservation", which, however, does not necessarily indicate the presence of concentration drift. Hence, explanation of concentration drift requires study of charge densities implied by, but not explicitly included in, the differential formulation (Sec. 3.1). Hund et al. (2001) further state that the differential and capacitor formulations are always equivalent. However, that is true only if the currents present in the model capture all movement of charge, which is not necessarily the case in, e.g., rapid equilibrium approximation (Sec. 3.3).

In this article, we showed that the capacitor formulation provides a physically consistent, well-defined formulation of membrane potential, and it avoids the problems all too often found in the differential formulation. In conclusion, we see little reason to use the differential formulation as the definition of membrane

potential in a spatially homogeneous myocyte model.

Acknowledgements

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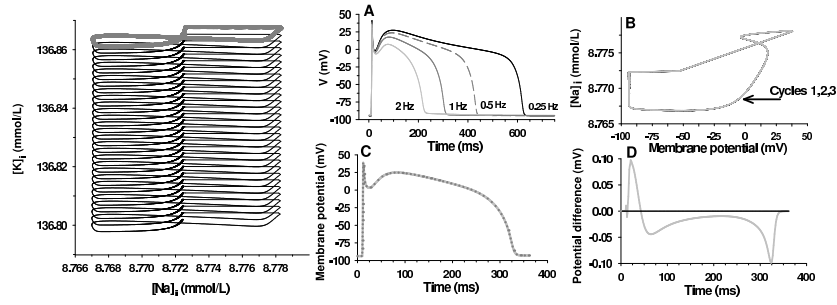
Figure 1: (A) When stimulus current is assigned to K^+ concentration in Case Study 1 model, the model has a limit cycle ("steady state"; grey cycle) in $([K]_i, [Na]_i)$ phase space. When the stimulus current is not assigned to any concentration, the model exhibits monotonic drifts away (black solid line) from the steady state; (B) Steady state action potentials at 0.25-2 Hz pacing rates in the Case Study 1 model. Membrane potential (ordinate; mV) is plotted against time (abscissa; ms); (C) Three simulations started from three different initial conditions (each with zero net charge) all approach the same steady state limit cycle in $([Na]_i, V)$ phase space in the Case study 1 model. Sodium concentration (ordinate; mmol/L) is plotted against membrane potential (abscissa; mV); (D) Action potentials simulated using the differential (solid grey line) and capacitor formulations (dashed dark grey line) and (E) the potential difference between the formulations in Greenstein et al. (2005, submitted to Biophys. J.) model.
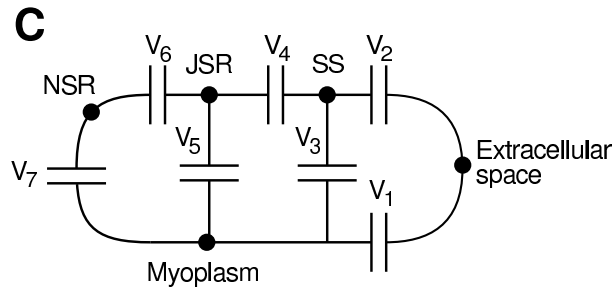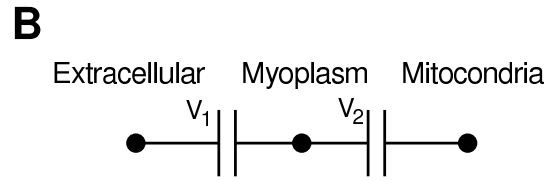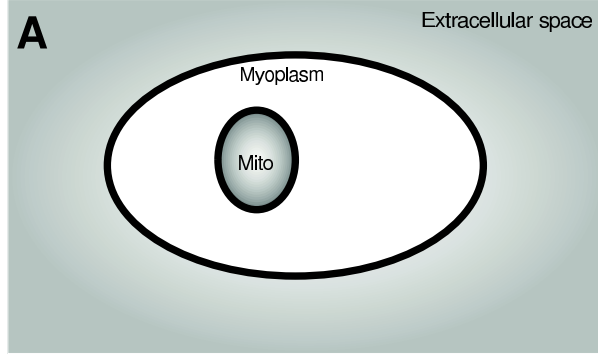
Figure 2: (*A*) Spatial representation and (*B*) capacitor representation of a myocyte model with three compartments: extracellular space, myoplasm and mitocondria; (C) Capacitor representation of five-compartment single-cell myocyte model, consisting of myoplasm, subspace (SS), junctional sarcoplasmic reticulum (JSR), network sarcoplasmic reticulum (NSR) compartments and an extracellular space. Membrane potentials between the compartments are denoted by V_k.
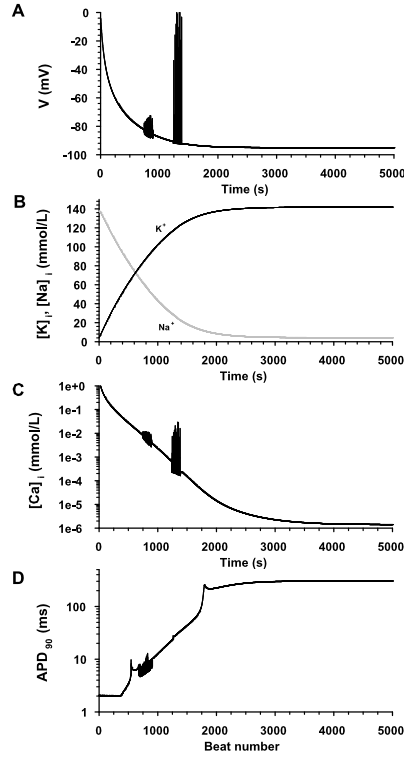
Figure 3: A Case study 1 simulation run demonstrating how a myocyte makes its way from a state with no concentration gradients to physiological steady state: (A) Resting membrane potential (ordinate; mV) sampled once a second, plotted against time (abscissa; s); (B) Sodium (gray) and Potassium (black) concentrations (ordinate; mmol/L) against time (abscissa; ms); (C) Diastolic calcium concentration (ordinate; mmol/L; logarithmic scale) plotted against time (abscissa; ms); (D) Action potential duration (ordinate; ms; logarithmic scale) plotted against time (abscissa; ms).
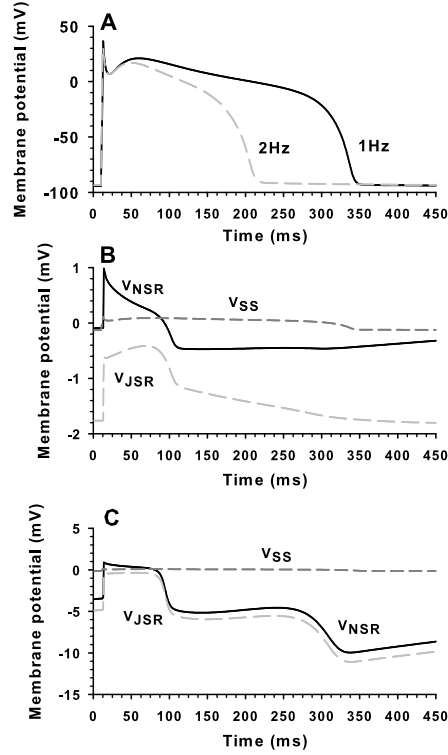
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Figure 4: Results from the Case study 2 model: (A) Shape of baseline action potential, paced at 1 Hz (solid black) and at 2 Hz (dashed grey). Membrane potential (ordinate; mV) is plotted against time (abscissa; ms); (B) Baseline membrane potentials (ordinate; mV) on myoplasm-JSR, myoplasm-NSR and myoplasm-SS interfaces plotted against time (abscissa; ms). (C) Increased membrane potentials (ordinate; mV) on myoplasm-JSR, myoplasm-NSR and myoplasm-SS interfaces plotted against time (abscissa; ms) in a simulation with reduced diffusion between intracellular compartments.